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6. AUTHOR(S)

RUSSELL R. BURTON, DVM, PHD

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

ARMSTRONG LABORATORY (AFMC)
CREW SYSTEMS DIRECTORATE
2509 KENNEDY CIRCLE
BROOKS AIR FORCE BASE, TEXAS 78235-5118

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13. ABSTRACT *(Maximum 200 words)*

Research using living systems, including whole living animals, has proved essential and invaluable in support of USAF operations. The USAF is aggressively seeking alternatives to animal research that we can integrate into our research programs. In the near future, we expect to make rapid advances in developing, as alternative cell culture techniques, mechanical surrogates and mathematical models. We will continue to use limited numbers of live animals in our research program, particularly where data from mathematical models are not reliable. But as we advance our techniques in using research animals with more and better chronic animal preparations, superior imaging methods and improved data extrapolation techniques, each animal study will require fewer laboratory animals. Of course, the human volunteer is the experimental subject of choice; thus, as non-invasive data collection techniques improve, the human will replace laboratory animals in even more of our biological research.

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COMMENTARY

Animals, Alternatives, and Humans: Solving Problems Through Research in the U.S. Air Force

RUSSELL R. BURTON, D.V.M., Ph.D.

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The Armstrong Laboratory and its predecessors have conducted responsible animal research in support of USAF operations for nearly three decades. The use of animal models is essential in research that requires a complex living system, but would be too hazardous to humans. The Laboratory also has aggressively pursued alternatives to the use of animals and improved methods in conducting animal research. Thus far, fewer animals are used currently in some areas of research. Since the human is the focus for Armstrong Laboratory research, human test subjects are used as frequently as possible. As improved noninvasive physiologic monitoring methods become available, humans will be used more extensively.

IN THE U.S. AIR FORCE (USAF), the human interacts somewhere in every weapon system; i.e., there are no unmanned systems. Thus, the optimal integration of the human into the weapons system is critical and presents unique human-related challenges. For that reason, one of four major USAF laboratories, the Armstrong Laboratory, is dedicated solely to an improved understanding of human capabilities and methods for enhancing them.

Consequently, the Armstrong Laboratory conducts most human-related research in the USAF. These experiments, designed to support human systems, frequently require living preparations: cell cultures, invertebrate animals, vertebrate animals including mammals, nonhuman primates, and when appropriate, the human. In conjunction with living tissues and whole organisms, alternative methods including mathematical and in vitro physical and chemical models are being developed by the USAF. These alternatives to animal use are proving to be effective in specific albeit limited areas of research. Use of humans, cell cultures, and animal-use alternatives eliminates the multiple disadvantages of non-human animal-use experimentation. These various research animal-use-related topics, along with improved use of research animals, will be discussed as they relate to our human-centered research and development (R&D) program.

Toxicology Research

The toxicology research program at the Armstrong Laboratory, which began in the early 1950's, has had two major objectives: 1) to identify the toxicity of hazardous chemicals and materials of interest to the USAF and DOD; and 2) to develop new techniques for accomplishing this objective that provide accurate and rapid risk assessment information (2,10). We are particularly interested in chemicals and toxic materials associated with advanced weapon systems. Knowledge of the toxic hazards involved in the development of advanced weaponry provides guidance for risk assessment decisions early in the acquisition cycle.

Each year, the USAF acquires several hundred new chemicals and materials. The cost associated with providing a complete toxicologic analysis of each of these chemicals is estimated to range from $500,000 to $1.5 million and requires 2 to 3 years. Traditional study methods also cost the lives of thousands of laboratory animals. Our research and testing programs have developed and implemented several methods that have greatly reduced the cost, time, and animal-use requirements.

Toxicokinetics and Pharmacodynamics Model

This program, which began in 1979, aimed to incorporate relevant physiological constraints and mathematical descriptors into computerized analytical models that predict human risk based on prior animal studies and various exposure scenarios (10). Using toxicokinetics (the knowledge of the absorption, distribution, me-

From the Armstrong Laboratory (AFMC), Crew Systems Directorate, Brooks AFB, TX.

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Address reprint requests to Dr. Burton, who is Chief Scientist of the Crew Systems Directorate, Armstrong Laboratory, 2509 Kennedy Circle, Brooks AFB, TX 78235–5118.

tabolism, and elimination of a chemical) in conjunction with the pharmacodynamics (the physiological effects caused by a chemical and its metabolites), mathematical models have been developed that are widely used in our toxicology program (Fig. 1).

These models have enabled us to accurately estimate human risk from existing animal data. They have also provided toxicologists a mechanism to predict the toxic effects of a chemical based on route of exposure. In addition, these models provide information useful in designing more efficient animal experiments, thereby significantly reducing the number and costs of animals required for testing.

Non-Invasive Technology Development

Nuclear Magnetic Resonance (NMR) technology uses resonance fingerprinting of several important biological elements to track the metabolism of a chemical within the body. This nondestructive-noninvasive technique, used in our laboratory, provides a method of repeat testing using only one animal (15). In addition, information gained from these advanced techniques has been helpful in improving the accuracy and predictive capabilities of our human-risk models.

NMR imaging is also used to follow the development of tumors in genetically cancer-prone mice after their exposure to chemicals. These animals serve as their own controls, thus reducing the number of animals used in each of these studies. Traditional cancer evaluation studies require control animals and several experimental groups to be sacrificed during the long course of a typical study.

In Vitro Testing Methods

The use of in vitro cell cultures was incorporated into our toxicology program in 1980. The liver is the primary site of chemical metabolism and biotransformation. Also, halogenated hydrocarbons that make up an important class of USAF chemicals target the liver. Therefore, over the past decade, we have developed an in vitro liver cell (hepatocyte) that approximates its in vivo state (11,12). That research has led to the successful development of in vitro hepatoxicity screens that rapidly determine the toxicity of halogenated fatty acids (13). Identification of metabolites from toxic chemicals in this manner is accomplished within 4 hours. In addition, methods developed in our laboratory for the isolation and culture of rat kidney cells have been equally successful in measuring the toxicity of hydrocarbon propellants. These data will be used to validate and expand our mathematical model.

A recent advance in this area of cell-culture testing involves fluorescent probes that have improved the sensitivity of these tests, thereby significantly reducing the number of cells required for each test. Also, fluorescent probes provide considerably more information on specific metabolic processes involving detoxification pathways and oxidative injury mechanisms. These in vitro probes have provided in detail cellular toxicities of specific cell reactions to known concentrations of a chemical upon reaching its intracellular target.

The use of cell cultures in toxicity analyses are compared with the traditional in vivo whole animal methods in Table I. Significant savings occur in numbers of animals used, time required, and amounts of chemical to be tested, but most impressive is the cost savings of 99.5% for testing 10 compounds.

Recent advances in in vitro testing systems have enormous potential for shortening the time requirements for carcinogenesis studies (2). Usually, carcinogenesis studies in animals require at least 2 years. Using DNA rapid growth techniques with flow cytometry, we can obtain screening genotoxic information in 4 d; e.g., specify protein expressions that are linked to mitogenesis.

These research and testing programs, which emphasize alternatives to animal experimentation, have significantly reduced our response time and animal costs. We now obtain greater accuracy with improved biological specificity, which provides more experimental flexibility. Hence, we are more responsive to operational requirements (our customers), as well as determining directions for future research.

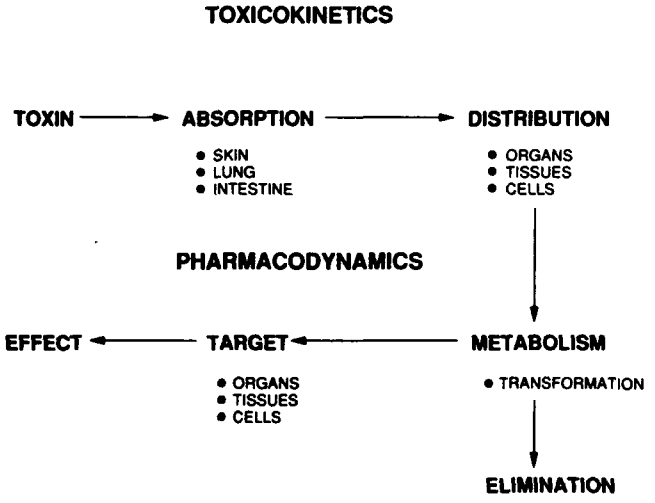

Fig. 1. Toxicokinetics and pharmacodynamics model design.

Predictive Models and Test Dummies in Biodynamic Research

The USAF is vitally interested in successful ejection of aircrew from aircraft, and their survival following

TABLE I. COMPARISON OF WHOLE ANIMAL METHODS WITH CELL CULTURE TECHNIQUES IN TOXICITY ANALYSES FOR 10 COMPOUNDS IN TRIPLICATE OR 30 COMPOUND TESTS.

	Whole Animal	Cell Culture
Number of animals required per compound test	20	1
Exposure time per test	2 Weeks	4 Days
Quantity of chemical for testing	14.0 gm	1.6 mg
Number of rats for 30 compound tests	600	3
Total rat cost	$8,400	$42.00 ($8,358 savings)
Study completion time per compound	6 Weeks	12 Days
Study completion time for 10 compounds	1.2 Years	0.3 Year

aircraft crashes. Two complementary techniques of analytical modeling and testing with dummies are used along with impact tolerance criteria to yield designs that provide maximum safety.

Computer based analytical models generate dynamic data of limb motions, accelerations and inertial forces, and harness restraint effects. For these models, input and validation data are obtained from human test subjects, research animals and, increasingly more important, the use of mechanical surrogates (anthropomorphic dummies) (Fig. 2). Our historical use of these mechanical surrogates began shortly before World War II, when bags of sand were used for weight/mass ballasting for ejection-seat testing. The USAF wanted more detailed and accurate information for their unique purposes, so we began to use the mechanical surrogates (dummies) that had been developed by General Motors (GM) for automobile crash analyses (14).

However, in the late 1960's, it became apparent that GM crash dummies could not meet the special requirements for data involving seat ejection research. An anthropomorphic dummy, "Dynamic Dan," with body response characteristics that were more similar to those of actual human bodies, was developed during 1968–71 (22).

Advances in technologies that replicated human joint biodynamic responses and improved instrumentation have resulted in the recent development of an even more life-like dummy called the Advanced Dynamic Anthropomorphic Manikin (ADAM) (3). This highly responsive model provides a human-like reactive live load for the ejection seat. It possesses realistic dynamics and kinematics in response to windblast, impact, and acceleration forces experienced during ejection (Fig. 3). Instrumentation within the manikin provides for extensive and rapid data acquisition, storage, and transmission.

In addition to ADAM, two predictive analytical models have been developed by the Armstrong Laboratory: 1) the Head-Spine model that predicts stresses of the spine due to abrupt accelerations applied to the torso primarily during ejection (4); and 2) the Articulated Total Body (ATB) model that predicts gross body dynamics primarily for crashworthiness and limb flail during ejection studies (9,21) (Fig. 4). These models are continually improving with more detailed data for humans and research animals.

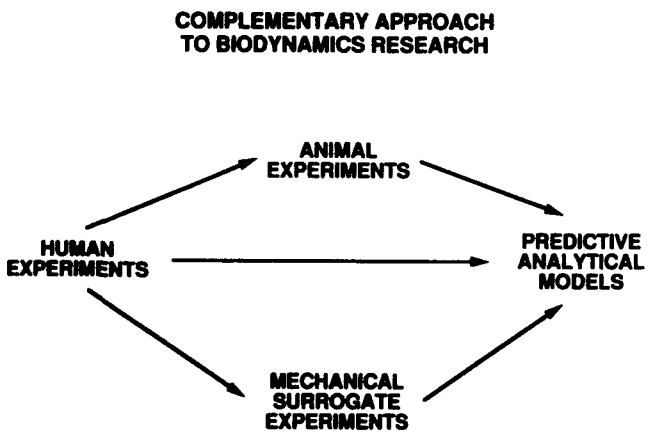

Fig. 2. Programmatic study design in biodynamic research.

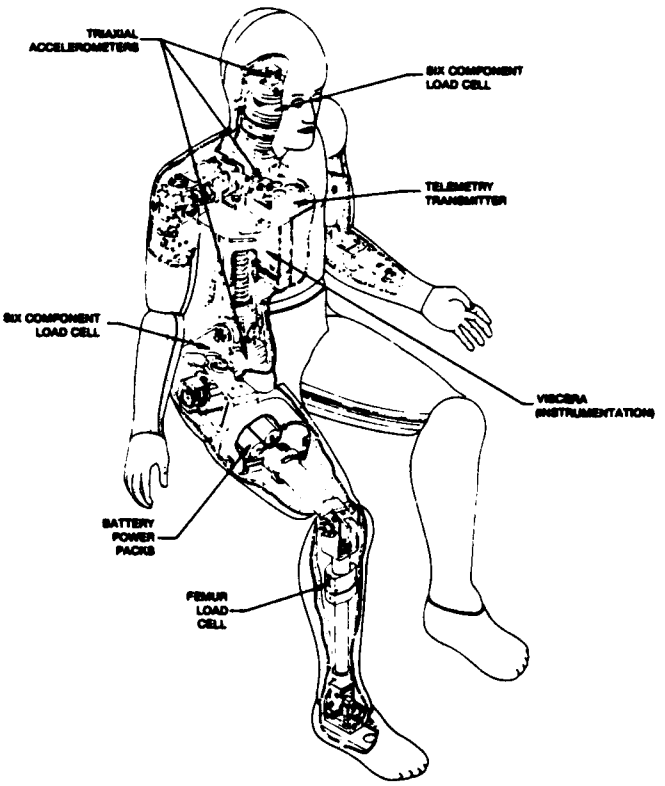

Fig. 3. Advanced Dynamic Anthropometric Manikin (ADAM) special features.

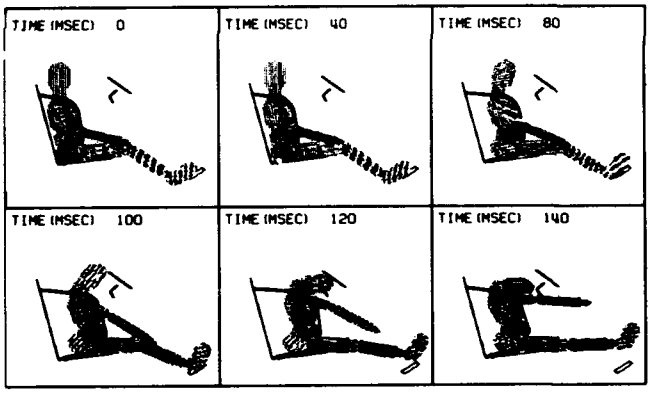

Fig. 4. Graphic representation of the Articulated Total Body (ATB) model during impact.

Burn Prediction Model

Human survival in aircraft fires is significantly enhanced if proper personal protective clothing is worn by aircrew. In determining levels of protection by material, four techniques are commonly used: 1) fire pit testing; 2) cloth flammability and thermal transfer measurements; 3) burn tests that involve laboratory animals; and 4) predictive models.

Fire pit testing uses instrumented fiberglass manikins for measuring maximum temperatures by moving the manikin through high intensity live fires. However, since the response of human flesh is not determined, anesthetized swine are used to measure the protection of different materials. Although this bioassay technique is quite accurate for selecting materials, it is much too

costly and time consuming, and it requires too many animals to be effective enough to screen many materials. Consequently, a model of burn mechanics was developed using a database from animal experimentation (19,20). This analytical model, called "BRNSIM," was developed in 1980 based on an earlier U.S. Navy model published in 1969. BRNSIM considers tissue water boiling, heat flow, blood flow changes and changes in the thermal properties of burning tissues. It accurately predicts depth of the burn in both humans and pigs. An example of the output of this model for pig burns is shown in Fig. 5.

This model can calculate protection coefficients of different materials using in vitro thermal transfer data to back-calculate the incident time-heat flux profile, and to predict burn depths. We continue to perfect this model to predict burn depth accurately under all circumstances. To evaluate four fabrics using the bioassay method requires 20 swine, 8–10 technicians and about 3 months. Our model accomplishes the same task with 1–2 technicians in 3 to 5 days without any experimental animals.

Live Fire Manikin Testing

The potential live fire hazards to aircrew are under study at the Armstrong Laboratory without using laboratory animals. These hazards include fragment strikes on the body, burns from fires and explosions, toxic effects from combustion gases, and blast overpressure effects on the auditory and pulmonary systems. To assess the degree of these hazards, ballistic shots are fired at an F-15 cockpit, and thermal, pressure and toxic fume measurements are made using state-of-the-art instrumentation. Fragment strikes are assessed by a newly developed fragment capture dummy called Aerospace Incapacitation Response Manikin (AIRMAN) (27). Fragment location, depth and track path obtained from the AIRMAN provide information for predicting crewmember wound type and severity using an analytical program called COMPUTERMAN (24,25).

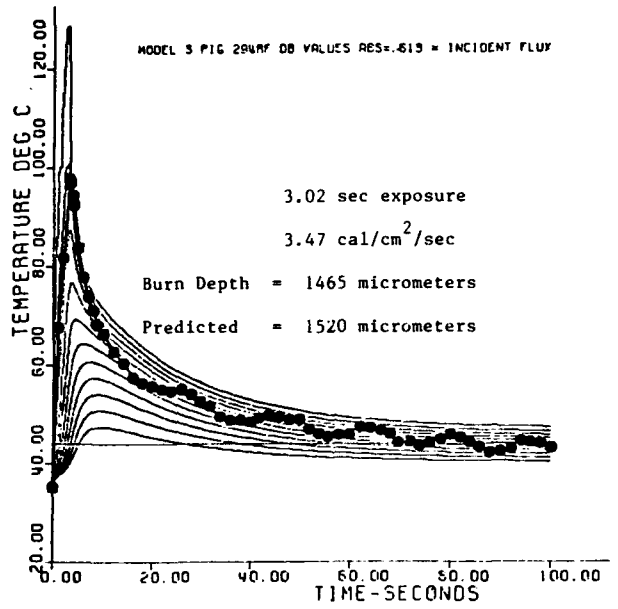

Fig. 5. Model output (solid line) simulating porcine burn data.

Radiofrequency, Electromagnetic, and Microwaves Radiation Research

We conduct research that establishes safety standards for electromagnetic (EMR), radiofrequency (RFR), and microwave, so-called non-ionizing radiations. Unable to detect physiologic and metabolic changes in animals at recommended safe levels, we are searching for any bioeffects that can be attributed to even lower exposure doses of radiation. These concerns led us to develop in vitro cell cultures beginning with studies in 1983.

In this type of difficult and extremely sensitive research, we believe that using cell culture technique has several advantages over laboratory animals. Using cell culture techniques, we can accomplish the following: 1) specify targets for toxicity or biological interaction (16,18); 2) design sensitive biological systems (18); 3) study cellular and intracellular molecular pathways (18); 4) limit the whole animal homeostatic protection mechanism—the isolated cell is more vulnerable (17); and 5) reduce the time, cost, and technical support required of research animals, even though in vitro research requires validation with whole animal data.

Animal-to-Human Extrapolation

Since all the animal-use experiments at Armstrong Laboratory are designed to support human systems, our ability to extrapolate data generated from animals to the human condition is of fundamental importance. This challenge is particularly great in animal-to-human extrapolation that involves psychological factors and performance skills, which are mediated by the central nervous system (1).

We have recently begun a research program to enhance our capability and confidence in relating nonhuman primate behavioral data to human applications. The basis for this program is to compare animal task results with human data obtained from tasks with the same levels of complexity. Experimental variables (e.g., drug effects) from both humans and animals can be directly compared so that higher dose results determined on animals can be extrapolated with more confidence to the human (Fig. 6). We believe that, as this research program progresses, performance effects on more complex human tasks can be more accurately interpreted, thereby increasing the utility of the animal data and decreasing the number of animal studies needed to answer a particular question.

Sustained High Acceleration Research

Remarkably, the human who routinely loses consciousness at acceleration forces of only 4 G can fly fighter aircraft successfully with acceleration profiles of 9 G, using modern G-protection methods (8). But the challenge to prevent G-induced loss of consciousness (G-LOC) remains, since completely safe G protection systems for USAF operations cannot be developed at this time. The bases for G-LOC involve the failure of brain physiologic and metabolic systems. Consequently, since a complete physiologically functioning

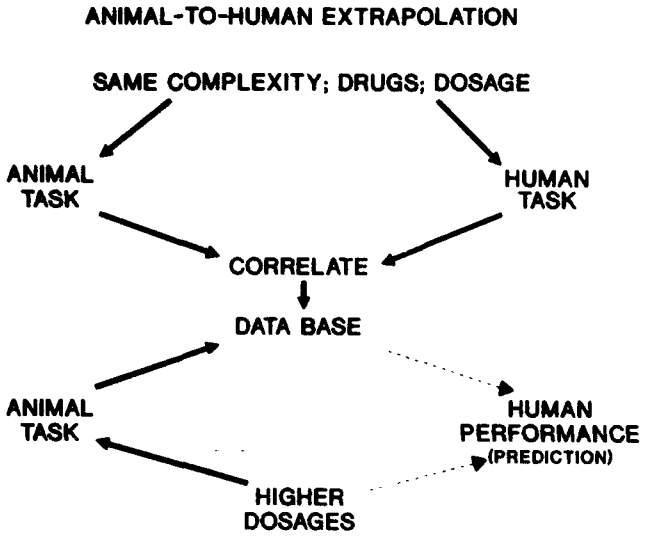

Fig. 6. Programmatic basis for animal-to-human extrapolation research.

organ is involved, live animals must be used in this research. Only limited human research can be safely conducted, and therefore rodent models have been developed (28). Animal research in this situation is closely related to human G-LOC research, since loss of consciousness appears to have a common physiologic basis.

We have developed the miniature swine and baboon as animal models for more physiologically sophisticated and less demanding sustained acceleration studies concerned with improving G-protection systems. Physiologic data from the swine compare favorably to data from humans exposed to G (5,7), and primate data are also used in G-LOC performance research (6). To increase the reproducibility of our research, we compare data from repeated exposures of the same animals with chronic instrumentation. These animal models are useful for several months as they remain healthy. Data obtained from these studies have been successfully translated to human requirements, resulting in significant advances in G protection methods that are now becoming operational in the USAF (8).

We are now developing a cardiopulmonary mathematical model, using baboons, that will be useful in validating future G-protection methods and cardiovascular disease relationships to G tolerance (26). We believe this model will eventually assist flight surgeons in determining the medical qualifications of fighter pilots for flying (23). Considering that each pilot is worth about $6 million in training costs, returning them to the cockpit is highly cost effective for the USAF.

Conclusion

Research using living systems, including whole living animals, has proved essential and invaluable in support of USAF operations. The USAF is aggressively seeking alternatives to animal research that we can integrate into our research program. We will continue to use limited numbers of live animals in our research program, particularly where data from mathematical models are not reliable. But as we advance our techniques in using research animals more effectively, each animal study will require fewer laboratory animals. Of course, human volunteers are the experimental subjects of choice, so as noninvasive data collection techniques improve, the human will replace laboratory animals in even more of our biological research.

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